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DOI: https://doi.org/10.1093/jac/36.3.513

Journal of Antimicrobial Chemotherapy (1995) **36**, 513–519

Brief reports

Effect of pO_2 and pH on synergy of tazobactam and β-lactam antibiotics against β-lactamase producing Enterobacteriaceae

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Synergy between tazobactam and ceftriaxone or piperacillin against β-lactamase producing Enterobacteriaceae was not influenced by the presence or absence of oxygen. For most strains synergy was excellent at neutral pH but reduced in acidic conditions. Low pH increased 50% of the MICs up to or beyond the sensitivity breakpoint.

Introduction

Resistance to β-lactam antibiotics due to the production of β-lactamases is common in Gram-negative and Gram-positive bacteria. One strategy to meet the challenge of β-lactamases was the development of non-hydrolyzable β-lactams. However, since their introduction and intensive use in clinical practice, an increasing number of previously unrecognised β-lactamases have been detected including inducible type I β-lactamases (Hennessey, 1967; Richmond & Sykes, 1973) or transferable plasmid-mediated extended spectrum β-lactamases with a wide range of substrate specificities (Kliebe *et al.*, 1985; Amyes, 1987).

The introduction of β-lactamase inhibitors was a novel strategy to overcome β-lactamase mediated resistance (Livermore, 1993). Clavulanate and penicillanic acid sulfones such as sulbactam and tazobactam are β-lactamase inhibitors which restore the efficacy of β-lactam antibiotics. However, the hydrolytic activity of some β-lactamases, particularly those of chromosomal origin, is not sufficiently inhibited by presently available inhibitors (Livermore, 1993). In addition, synergy between the β-lactam and β-lactamase inhibitor may be compromised by phenotypic pathogen resistance due to variable conditions at the site of infection (Livermore & Corkill, 1992).

Local factors such as pO_2 and pH may differ significantly from conditions used in standard in-vitro susceptibility testing (Simmen & Blaser, 1993). Modified culture conditions during these tests e.g. acidic pH or anaerobiosis, which differ from NCCLS standards (NCCLS, 1990) but simulate pathological conditions in some infections may reduce the efficacy of several antibiotics, e.g. aminoglycosides or quinolones (König, Simmen & Blaser, 1993). Livermore & Corkill (1992) have shown that acidic pH reduces synergy between β-lactams and β-lactamase inhibitors against β-lactamase producing *Escherichia coli*. The purpose of this study was to examine various pathogens and

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0305–7453/95/090513 + 07 $12.00/0

β-lactamases and determine whether anaerobic culture conditions impair synergy between tazobactam (TAZ) and β-lactams and further limit synergistic interaction at acidic pH.

Materials and methods

Bacteria and β-lactamases

Strains of *E. coli* and *Klebsiella pneumoniae* with different β-lactamases were used: *E. coli* CF102 (TEM-3 β-lactamase), *E. coli* HB101 (TEM-4), *E. coli* JC2926 (SHV-2), *E. coli* W3110 (SHV-2), *E. coli* X53-2 (SHV-4), *K. pneumoniae* CF104 (TEM-3), *K. pneumoniae* CF504 (TEM-5), *K. pneumoniae* B7368 (SHV-2). In addition, two strains with type-I β-lactamases, *Citrobacter freundii* 1982 and *Enterobacter cloacae* 908R, were included.

Antibiotics

The β-lactamase inhibitor tazobactam was combined with either ceftriaxone or piperacillin. Tazobactam concentration was 8 mg/L in all experiments with piperacillin/tazobactam and in most experiments with ceftriaxone/tazobactam. In experiments with ceftriaxone against the highly sensitive strains *E. coli* CF102 and *K. pneumoniae* CF104, the concentration of tazobactam was reduced from 8 to 1 mg/L.

MIC determination under standard and modified culture conditions

All MICs were determined simultaneously under standard and modified culture conditions using a 2 mL macrodilution assay in cation-adjusted Mueller-Hinton broth with an inoculum of $1–5 \times 10^5$ cfu/mL. Four different sets of culture conditions were tested: (*i*) *standard culture conditions*: MICs were determined according to the NCCLS protocol (document M7-A2, 1990) in air in neutral MHB-S (pH 7·3); (*ii*) *aerobic-acidic culture conditions*: MICs were determined in a BBL jar, in a CO_2-enriched atmosphere (BBL GasPak CO_2 Systems, CO_2 generator envelopes, BBL Microbiological Systems, USA) resulting in acidification of the broth to pH 6·4; (*iii*) *Anaerobic-acidic and* (*iv*) *anaerobic-neutral conditions*: MICs were determined in a BBL anaerobic jar (BBL GasPak Plus Anaerobic System, H_2-CO_2 generator envelopes) in unbuffered broth for anaerobic-acidic conditions and in NaOH-buffered broth for anaerobic-neutral conditions. Susceptibility testing under standard and modified culture conditions was performed as previously described (König *et al.*, 1993). MIC determinations for each strain and both β-lactam-TAZ combinations were done in triplicate to obtain a median MIC. To quantify the extent of synergy, ratios of the MIC of the β-lactam alone divided by the MIC of the β-lactam in combination with TAZ were calculated.

Results

Synergy at standard culture conditions

Tazobactam proved to be a very potent inhibitor of β-lactamase activity for most tested strains with plasmid encoded β-lactamases. All MICs of ceftriaxone and piperacillin were considerably higher than the respective susceptibility breakpoints of 8 and 16 mg/L (NCCLS, 1990). The addition of tazobactam reduced the MICs to or below

the respective breakpoints except for two strains of *Klebsiella* (Tables I and II). Median ratios were 512 for $MIC_{CRO}/MIC_{CRO\,\&\,TAZ}$ and 128 for $MIC_{PIP}/MIC_{PIP\,\&\,TAZ}$. The synergy was particularly impressive for the combination of ceftriaxone/tazobactam. On average, the enhancement of activity of ceftriaxone by tazobactam was eight-fold greater than for piperacillin/tazobactam irrespective of the type of plasmid encoded β-lactamase ($P < 0{\cdot}05$). In addition, the MICs of ceftriaxone/tazobactam were 64-fold lower than the corresponding MICs of piperacillin/tazobactam at neutral pH.

However, the pronounced enhancement of the β-lactam activity was not a consistent finding. While synergy was excellent for the *K. pneumoniae* strain CF104 expressing a TEM-3 β-lactamases, it was rather limited against the two strains *K. pneumoniae* CF504 and B7368 with TEM-5 and SHV-2 type β-lactamases, respectively. For both strains, the MIC of piperacillin of >512 mg/L was reduced to 512 mg/L in the presence of tazobactam. MICs of ceftriaxone of 64 mg/L and 128 mg/L were reduced eight- and 16-fold by tazobactam for *K. pneumoniae* CF504 and B7368, respectively.

Synergy between tazobactam and ceftriaxone or piperacillin was also rather limited for strains with chromosomal β-lactamases. By adding tazobactam, the MICs of ceftriaxone and piperacillin were reduced four-fold from 512 to 128 mg/L for the strain *E. cloacae* 908R. With *C. freundi* 1982, the MIC of ceftriaxone was reduced by tazobactam 16-fold, from 64 mg/L to 4 mg/L.

Synergy at modified culture conditions

Anaerobic culture conditions did not compromise synergistic interaction between tazobactam and ceftriaxone or piperacillin. The median ratios of $MIC_{CRO}/MIC_{CRO\,\&\,TAZ}$ were 512 at both, aerobic-neutral and anaerobic-neutral culture conditions, the corresponding ratios for piperacillin being 128. In contrast, acidic pH reduced the synergy of β-lactam-tazobactam combinations. The median ratio of $MIC_{CRO}/MIC_{CRO\,\&\,TAZ}$ of 512 (range: 8–2048) under standard culture conditions was reduced to 8 in aerobic-acidic and anaerobic-acidic (range: 1–256 and 1–128, respectively) culture conditions. Similarly, the median ratios for piperacillin and tazobactam were reduced from 128 (32–256) under standard conditions to 32 (1–128) in aerobic-acidic and to 16 (1–64) in anaerobic-acidic conditions. These findings showed that, in contrast to the marked effect of acidic pH, the presence or absence of oxygen had no further significant effect on the synergy.

The extent of this reduction varied with strain, β-lactam and type of β-lactamase. Acidic pH had a weakly antagonistic effect on piperacillin MICs against TEM-3, however the corresponding ceftriaxone MICs showed a stronger effect. The extent of synergy impairment varied with the type of β-lactamase. Synergy against TEM-3 and TEM-5 was weakly compromised but against TEM-4 and SHV-2 strongly so and against SHV-4 severely compromised. The antagonistic effect of acidic pH on synergistic interaction with tazobactam tended to be slightly more pronounced (two-fold) for ceftriaxone than piperacillin.

In summary, the extent of the reduction of synergy was at least 16-fold in more than half of the experiments with acidic pH. As a result of compromised synergy, 50% of the MICs determined at pH 6·4 were above the NCCLS susceptibility breakpoints for ceftriaxone and piperacillin.

Table I. Median (range) of standard MIC or ceftriaxone and of standard and modified MICs of the ceftriaxone/tazobactam combination determined in triplicate.

Strain	Culture condition: β-Lactamase	MIC of ceftriaxone (mg/L) standard	MIC of ceftriaxone/tazobactam (mg/L) standard	aerobic-acidic	anaerobic-acidic	anaerobic-neutral
E. coli CF102[a]	TEM-3	64 (32–128)	0·06 (0·06)	0·25 (0·125–0·25)	0·5 (0·25–1)	0·06 (0·06)
K. pneumoniae CF104[a]	TEM-3	128 (64–256)	0·25 (0·125–0·5)	8 (4–16)	2 (2–4)	0·25 (0·125–0·25)
K. pneumoniae CF504	TEM-5	64 (32–64)	8 (8)	32 (32–64)	32 (32–64)	2 (2)
K. pneumoniae B7368	SHV-2	128 (64–128)	8 (4–16)	64 (64–128)	64 (64)	4 (2–8)
E. coli HB101	TEM-4	256 (128–256)	0·125 (0·125–0·5)	16 (8–16)	4 (2–8)	0·06 (0·06–0·125)
E. coli JC2926	SHV-2	16 (8–16)	0·06 (0·06–0·25)	2 (0·5–4)	1 (0·25–2)	0·06 (0·03–0·125)
E. coli W3110	SHV-2	32 (32)	0·125 (0·06–0·125)	8 (4–8)	8 (4–8)	0·125 (0·06–0·125)
E. coli X53-2	SHV-4	32 (32–64)	0·06 (0·03–0·125)	32 (16–32)	32 (16–32)	0·06 (0·06–0·125)

[a]Concentration of TAZ was 1 mg/L for experiments with the strains *E. coli* CF102 and *K. pneumoniae* CF104, and 8 mg/L for all other experiments.

Table II. Median (range) of standard MIC of piperacillin and of standard and modified MICs of the piperacillin/tazobactam combination determined in triplicate

Strain	Culture condition: β-lactamase	MIC of piperacillin (mg/L) standard	MIC of piperacillin/tazobactam standard	aerobic-acidic	anaerobic-acidic	anaerobic-neutral
E. coli CF102	TEM-3	256 (256)	1 (1–4)	2 (2)	4 (2–4)	2 (2)
K. pneumoniae CF104	TEM-3	512 (512)	8 (8–16)	16 (16)	16 (8–16)	8 (8)
K. pneumoniae CF504	TEM-5	>512 (>512)	512 (256–512)	>512 (>512)	>512 (>512)	128 (128)
K. pneumoniae B7368	SHV-2	>512 (>512)	512 (256–512)	n.d.	>512 (>512)	512 (≥512)
E. coli HB101	TEM-4	>256 (>256)	2 (2–4)	8 (8)	32 (32)	n.d.
E. coli W3110	SHV-2	>256 (>256)	16 (4–32)	512 (512)	512 (256–512)	8 (8–16)
E. coli X53–2	SHV-4	>256 (>256)	4 (2–8)	>512 (>512)	>256 (>256)	4 (4)

Discussion

Previous studies with β-lactamase non-producers have shown that the antimicrobial activity of cephalosporins and penicillins is neither reduced by acidic pH nor by anaerobic conditions (Livermore & Corkill, 1992; König *et al.*, 1993). In contrast, Livermore & Corkill (1992) have shown that the inhibitory interaction of the sulfones tazobactam and sulbactam with extracted TEM-1 enzyme was pH dependent. This study confirms these findings and documents for a wider range of organisms and β-lactamases the compromising effect of acidic pH on synergy. The antagonistic effect of acidic pH is quite heterogeneous within the TEM β-lactamases. Little effect was observed with TEM-5 and TEM-3, whereas a pronounced effect was found with TEM-4. Livermore & Corkill also reported little effect with TEM-3 and pronounced effects with TEM-1 and TEM-2.

In contrast to the marked effect of variations in pH, the presence or absence of oxygen did not further modify the activity of the β-lactamase inhibitor.

These findings may have clinical relevance for the treatment of a number of infections where acidic pH prevails; for example pH values of $<6{\cdot}5$ have been frequently documented in intra-abdominal infections and abscesses (Simmen & Blaser, 1993) or purulent airways (Bodem *et al.*, 1983). Similarly, the endobronchial pH is acidic (6·5–6·7) in healthy persons and in patients with chronic lung diseases and pneumonia. In-vivo experiments and clinical investigation of infections involving sites with acidic pH should show whether combinations of β-lactam antibiotics with the β-lactamase inhibitor tazobactam are also effective against β-lactamase producing pathogens.

Acknowledgements

We thank Dr R. Then, Basel, Switzerland, and Prof. B. Wiedemann, Bonn, FR Germany, for providing the β-lactamase producing strains.

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(*Received 15 November 1994; returned 12 December 1994; revised 9 March 1995; accepted 13 April 1995*)